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学位論文要旨

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題名： **Alpha-actinin and desmin: two proteins that constitute Z-line of myofibrils**

（アルファーアクチニンとデスミン：筋原線維の Z-線を構成する二つのタンパク質）

In order to examine the molecular organization of the Z-line of a sarcomere, we focused on two proteins; alpha-actinin and desmin. As mentioned above, alpha-actinin is a class of actin-bundling proteins and is attributed to the major component of the Z-filaments. Desmin, on the other hand, forms three dimensional network around myofibrils anchoring on the surface of Z-line. The papers consists of two parts. In Part I, we investigated the conformation of alpha-actinin with special regards to the characteristic domain structure of alpha-actinin.

Part I Conformational changes of alpha-actinin induced by binding to F-actin

Alpha-actinin cross-links actin filaments both in muscle and non-muscle cells. Alpha-actinins consist of three major functional domains; N-terminal actin-binding domain (ABD), central rod domain that is responsible for the homo-dimer formation and C-terminal regulatory domain. The linker domain between the actin-binding domain and the rod domain is flexible, allowing the actin-binding domain to assume various orientations to form different types of networks with actin filaments. The linker region is sensitivity to the proteolytic cleavage. Limited proteolysis of alpha-actinin results in a number of protease resistant fragments representing the actin binding domain and the rod domain

In order to examine the effect of actin binding on the conformation of alpha-actinin, we carried out limited digestion of alpha-actinin by chymotrypsin in the presence and absence of F-actin. It was shown that 50 % of the 105 kDa-subunit was digested after 10 hours of digestion in the presence of F-actin, while 85 % was cleaved in the absence of actin. Formation of 32 kDa-head and 55 kDa-rod domain were also retarded when alpha-actinin-F-actin complex was digested. Although F-actin affected the rate of the alpha-actinin digestion by chymotrypsin, the digestion to 55 kDa-rod and 32 kDa-head domains proceeded through common intermediate products with chain molecular weights of 98 k, 87 k, 72 k, and 68 k. N-terminal sequencing of 55 kDa-fragment showed that the neck region was cleaved at 276-Leu. The cleavage site was not affected by binding to F-actin nor ionic strength of the solvent. It was also indicated that α-actinin was cleaved at 15-Tyr by chymotrypsin. Assignment of cleavage products on the primary sequence of chicken skeletal muscle alpha-actinin showed that there are thee major cleavage sites on native alpha-actinin by chymotrypsin. Quantitation of cleavage products in the presence or absence of F-actin indicated that the cleavage site III located between the rod-domain and regulatory domain was affected by the binding of F-actin. It

is suggested that interaction of the neck region of one subunit of the anti-parallel dimer of with the C-terminal regulatory domain of the opposite subunit.

Further to examine the conformation of alpha-actinin, the surface accessibility of the cysteine residues of alpha-actinin to the thiol-targeted reagent 7-dimethylamino-4-methyl-counmarinyl) maleimide) (DACM) was investigated. DACM labeled three cysteine residues per 105 kDa-subunit of alpha-actinin. Chymotryptic digestion of DACM-alpha-actinin indicated that 32 kDa- head domain which was detected on the CBB stain was not detected on the UV light. Cysteines in head domain were completely inaccessible and presumably were buried. Therefore, three exposed cysteine residues located in the rod domain and/or regulatory domain of alpha-actinin.

Binding activity of DACM-labeled alpha-actinin was examined by pelleting method. The binding curve of DACM-alpha-actinin to F-actin was quite similar to that of control α-actinin and gave a saturated value when one alpha-actinin dimer binds to 14 G-actin. The apparent dissociation constant (Kd) of DACM-α-actinin also showed the similar value to that of unlabeled α-actinin. Therefore, we concluded that modification of three cysteins residues on actin binding head domain of alpha-actinin did not affect the bundling activity of alpha-actinin. Gelation ability of DACM-alpha-actinin was investigated by the miniature falling ball assay. In falling ball experiment, alpha-actinin induced the gelation of alpha-actinin-F-actin solution at the 25 °C. On the other hand, DACM-alpha-actinin-F-actin did not induce the gelation of F-actin. These observations indicated that gelation and binding activity of alpha-actinin are regulated independently. Furthermore, these results suggested that conformational change of the rod-domain affected the activity of actin binding domain.

Part II　Assembly properties of desmin measured by flow birefringence

Desmin is a muscle specific subunit of intermediate filaments. Desmin filaments encircle Z-lines of striated muscle and hold the striated pattern of adjacent myofibrils in registered. When compared with the other two cytoskeletal filaments, actin filaments and microtubules, molecular architecture and assembly properties of intermediate filaments are less understood . We have investigated the flow birefringence property and assembly process of desmin. Solution of non-polar desmin filaments showed birefringence when aligned in the sheared flow. The amount of birefringence of desmin filaments was considerably lower when compared with that of F-actin solution. Assembly of desmin from soluble state was followed by the birefringence measurements. At any desmin concentrations examined, the degree of flow birefringence increased rapidly just after the addition of the assembly buffer and reached a saturated level within 30 min. The time to reach half-maximal values of flow birefringence slightly but definitely depended on the initial soluble desmin concentrations. The plotting of the initial velocity of the assembly against the soluble desmin concentrations showed a slope of 1.4. This result suggested that the assembly process detected by flow birefringence measurements followed second-order kinetics, and the process corresponded to the second step of the three stage model for type III intermediate filament assembly proposed by Herrmann and his colleagues; the annealing of unit length filaments into filaments.